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PROGRAM TITLE:	SMOKE CONDENSATE STUDIES
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A. SALT EFFECTS (with 6906)

The level of filler potassium was lower than expected[1] for the salt sprayed RL base web samples and the control which were prepared last month.[2] Rather than proceeding to base fraction studies as planned, other analytical data were evaluated and a few handmade cigarettes were prepared and smoked.[1,3] The delivery of the KOAc sprayed base web cigarettes was half that of the control, while the KCl sprayed sample showed an intermediate delivery, in line with previous results. The activity of these IT CSCs are also being evaluated in the *Salmonella*/microsome assay before the larger scale base fraction preparation is conducted.

B. MW 288[4]

Study of the fractions isolated from the thermal decomposition products of α-4,8,13-duvatriene-1,3-diol continued.[2] A MW 288 aldehyde of unknown structure is present in one fraction. Further studies are planned to elucidate the structure of this aldehyde.

C. NITROSAMINES[5]

Acceptable gc column performance for the nonvolatile nitrosamine analysis was reestablished. A number of RL samples were analyzed for nonvolatile nitrosamines in filler and smoke. Work is also progressing on a survey of filler types for nitrosamine deliveries to smoke. Cigarettes have been obtained for an initial evaluation of dilution and filter effects on both volatile and nonvolatile nitrosamines in smoke.

D. PAH PROCEDURE[6]

Either EM Si-60 or Si-40 silica gel column chromatography was found to be satisfactory for the first step in the PAH isolation procedure. Florisil PR 60/100 will probably be used for the third step but some problems with selective PAH removal were encountered. It is expected that some additional optimization of the activation conditions and the elution solvent composition will give a satisfactory overall procedure.

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A Jennings bonded phase capillary gc column is being evaluated using the HP on column injector. Both the on column injector and the column have performed well for the PAH analysis.

E. CHROMATOGRAPHY AND GC/MS

A 600 mg sample of X6D3IM IT CSC was used to evaluate the potential applicability of the Chromatotron® system to smoke condensate fraction studies. Despite problems due to the complexity of CSC, this rapid preparative chromatographic procedure appears to be well suited to present and planned fractionation studies.[7]

A gc/ms analysis of air contaminants was conducted as part of a collaborative assistance to the Manchester Fire Department.[8,9] Several fractions were also examined by gc/ms as part of our studies of carbolines in the base fraction of smoke. A major problem with these studies is the lack of components amenable to either packed or capillary gc.[8,9] The availability of a "stand alone" Sigma 3 gc, as part of the overall new VG MM16 gc/ms system, should provide the opportunity for more developmental chromatographic studies. We expect to transfer the current du Pont 21-490 gc/ms/ds to Chemical Research and to install the new system during September.[8,9]

F. ACTIVE BASE FRACTION COMPONENTS (with 6906)

A larger sample of the X6D3IM base fraction (50% methanol extract) was chromatographed on Sephadex LH-20. There were some small differences from the previous small scale experiment and from the three large scale separations of the toluene extract on LH-20.[2] However, the 50% methanol extract fractions continued to show enhanced accountability, indicating the potential activity of the parent fraction is diminished by some interactive effects among its components.[10]

Efforts to determine if the active carbolines, Trp-P-1 and Trp-P-2, are present at significant levels in CSC continued this month. These are produced from the pyrolysis of tryptophan, but the low level of this amino acid in filler made it difficult to establish if any Trp-P-1 or Trp-P-2 was present in X6D3IM CSC.[2]

A LTF-2A filler with added tryptophan (1% filler nitrogen) was selected as an optimal test case for Trp-P-1 and Trp-P-2 formation during smoking. Before beginning the experiment, the cleanup procedure was optimized using a mixture of Trp-P-1, Trp-P-2, harman, and norharman. The first step was silica gel column chromatography of the base fraction. The next step was tlc under carefully controlled conditions. Final cleanup used RP-HPLC with fluorescence detection. A 2.1 g sample of IT CSC was used for the actual experiment. Neither Trp-P-1 nor Trp-P-2 were detected after fractionation of this sample. Based on the detection limits of the

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procedure, Trp-P-1 and Trp-P-2 (if present at all) cannot make a significant contribution to CSC activity.[11] We plan to conclude this study in the near future based on these results.

Studies directed at determining if Glu-P-1 and Glu-P-2 (active pyrolysis products of glutamic acid) are present in burley base fraction continued.[12] The first step, as in the Trp-P-1 study, was silica gel chromatography of the base fraction. A silica gel TLC procedure was developed for the next step. For preparative TLC, $CHCl_3$/acetone (4/1) with multiple development of the plates, gave satisfactory results. The enriched fraction obtained in this manner is now being used for RP HPLC profiling.

G. PYROLYSIS (with 6906)[3,13]

The evaluation of the activity of tobacco pyrolyzates continued with a study of bright and burley fillers heated in nitrogen. Samples were prepared over the 400° to 800°C range at 100° increments. As usual, data related to weight loss and pyrolyzate collection were obtained. The *Salmonella*/microsome assay results for these samples showed: the burley pyrolyzate was more active at all temperatures than the bright pyrolyzate; burley produced optimal activity at 500°, while bright produced optimal activity at 700°. These results will be reconfirmed and extended in studies now in progress. The first samples will explore the effects of He *versus* N_2 as pyrolysis atmosphere and the effect of extensive purging of the filler with either gas prior to pyrolysis.

H. SIDESTREAM SMOKE STUDIES (with 6906)[3]

A series of sidestream smoke samples were prepared from Kentucky reference, RCB, burley, bright, and RL reference cigarettes. Included in the evaluation were various trapping procedures, static *versus* dynamic burn, and mainstream samples for reference. The assay results are reviewed in detail in the current 6906 Progress Report. This experiment provided the basis for more extensive studies which will use TPM since both MS and SS samples can be obtained from the same cigarettes during dynamic burn. Experiments are now in progress to evaluate the procedure to be used for TPM preparation for these studies.

I. BURLEY CSC FRACTIONATION (with 6906)[1]

Experiments were designed to evaluate the activity of smoke fractions more completely in the yeast assay. Burley IT CSC was used for acids, bases, neutrals, and residual water solubles fractionation. In addition, a water-ethyl acetate partition was conducted. Further fractionation will depend on the results of the biological evaluation of these samples.

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